Correlation Between Response to Chemotherapy of Human Tumors in Patients and in Nude Mice

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Human tumors serially heterotransplanted in nude mice have been tested for their response to chemotherapeutic agents. Fourteen melanomas, 14 colorectal carcinomas, and 14 breast carcinomas have been used. Each tumor originated in a different patient. The tumors were maintained by serial subcutaneous transplantation in nude mice. For the experiments in this study, each neoplasm was transplanted under the kidney capsule of 60 to 100 adult nude mice. The areas of the individual tumor implants were precisely measured immediately after insertion using a stereo microscope equipped with a micrometric ocular. The animals were then divided into groups of six to ten animals each. One group was injected daily with saline and served as controls. The mice in the remaining groups were injected daily for eight days with one of the following chemotherapeutic agents—Adriamycin (doxorubicin), 5-fluorouracil, methotrexate, Cytoxan (cyclophosphamide), Alkeran (melphalan), vincristine, vinblastine, methyl-CCNU, or BCNU—at optimum doses (the maximum dose tolerated that causes less than 10% weight loss). Treatment was initiated when the implants were well established, having roughly doubled their initial mass. The animals were then sacrificed and the tumors measured again. A drug was rated effective only if it inhibited growth of the tumor by 99% or more. The results so obtained were compared with the published results of various clinical trials. When the sensitivity of the human tumors in the mice was compared with the sensitivity of tumors of the same type that had been treated in human patients, a close correlation was found. The panel study detected nine of ten effective drugs, giving only two false-positive results. Our data strongly support the validity of heterotransplants of human tumors in the nude mouse as a predictive system for testing new anticancer agents and in determining optimal treatment schedules and combinations of known drugs.

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In recent years, human tumors heterotransplanted in immunosuppressed animals, particularly nude mice, have been widely used to assess the antitumor activities of various chemotherapeutic agents. Such model systems have been most often used to find new anticancer chemicals.[1–3]

A large body of results is accumulating. However, the validity of such results depends upon the reliability of the model employed. In other words, does a particular tumor treated with a given chemical respond in the same way in the patient and in the nude mice? A valid answer to this question can be obtained in two main ways. The first, and most direct, method consists of treating human malignancies with an anticancer agent in the patient of origin and as heterotransplants growing in the nude mice. By comparing the results obtained on the same tumor in the two systems, it is possible to assess the similarity of results and determine to what extent human results can be predicted from results in the mice. We are conducting such a study; however, it is necessary to wait for the assessment of clinical results in the patient (for example, the reappearance of a removed neoplasm) before this comparison can be made. The second method, postulated by Bellet *et al.*,[4] consists of assessing the responses to a given anticancer agent of a panel of human tumors of the same histologic type that have been heterotransplanted in nude mice. If the response of human

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TABLE 1. Melanomas

umor	Sex/age	Location	Melanoma: histologic type	Previous treatment	Nude passages	Melanin	Refs.
EBE	F/49	Left lung	Metastatic	AL, BCG, *C. parvum*, DTIC	6	A	
VIL	F/30	Right forearm	Recurrent	None	25	A	
FRA	M/43	Left thigh	Recurrent	None	7	M	
STE	F/32	Femoral lymph node	Metastatic	AL	5	A	
BOW	M/36	Skin of back	Recurrent	BCG, *C. parvum*	29	A	
HOF	F/50	Skin of leg	Recurrent	BCG, AL	20	A	(17)
NIS	M/54	Lymph node, neck	Metastatic	None	15	M	
FOS	F/54	Iliac lymph node	Metastatic	None	11	A	(17)
TRI	F/45	Skin of shoulder	Primary	None	14	A	
VIC	M/38	Left thigh	Metastatic	Rx	1	A	
BAG	M/21	Right foot	Primary	None	6	A	
GIL	M/55	Left groin	Metastatic	AL, *C. parvum*, DTIC	12	A	
FIN	M/51	Axillary lymph node	Metastatic	BCG, Rx	4	A	
BRO	M/25	Left shoulder	Primary	None	25	A	

A: amelanotic; M: melanotic; AL: Alkeran (melphalan); BCG: Bacillus Calmette Guerin; DTIC: dacarbazine; Rx: radiation therapy.

umors of the type being studied is known, the response f the mice can be compared to the human response. y statistical methods, such correlation can be quantiated. In this article, we are reporting our results using his second approach. Gehan[5] has demonstrated that if t least one in a panel of 14 tumors of the same type esponds to a given treatment, there is a 95% probability hat 20% or more of such tumors will respond to the gent in question. We have used three panels of 14 tuhors each: one panel of melanomas, one of colorectal arcinomas, and one of breast carcinomas.

Materials and Methods

umors

The tumors used for these experiments are all human umors, the majority of which were obtained by heterotransplanting human bioptic material directly from the patient to the nude mice. The origins and main characteristics of each tumor are summarized in Tables 1 through 4.

Animals

Swiss nude mice bred and maintained in our laboratory under pathogen-free conditions[6] were used throughout this study. The average age of the mice used in the experiments was 3 months.

Experimental Procedure

Human tumors serially transplanted in nude mice were used. Small fragments of each tumor were transplanted under the kidney capsules of nude mice using our modification of the technique of Bogden *et al.*[1] Each

TABLE 2. Colorectal Carcinomas

umor	Sex/age	Location	Histologic type	Previous treatment	Nude passages	References
GOB	F/63	Cecum primary	Mod diff adeno, cecum	None	20	
CLO	F/64	Liver metastasis	Mod to poor diff adeno, rectum	None	13	
EI	?/?	?	Mod diff adeno	?	6	
EY	M/29	Rectum primary	Mod diff adeno, rectum	None	19	
CEN	F/83	Colon (cell line) primary	Very poorly diff adeno	None	34	(18, 19)
HT-29	F/44	Colon primary	Mucinous (mod to poor diff) adeno, colon	?	32	(20)
EG	M/60	Colon primary	Mucinous (mod diff) adeno, colon	None	16	
QU	M/50	Diaphragm metastasis	Mucinous (mod diff) adeno	?	16	
CON	F/41	Ovary metastasis	Mod to poor diff adeno, colon	5FU	8	
AS	M/50	Colon (cell line) primary	Poorly diff adeno	None	7	(19)
NZ	F/81	Colon primary	Mod to poor diff adeno colon	None	11	
IOR	F/70	Liver metastasis	Mod diff adeno, colon	None	4	
IOV	F/64	Femoral lymph node	Poorly diff adeno rectum	None	3	
VAL	F/48	Vaginal spread	Anorectal squamous cell ca	None	9	

Mod: moderately; diff: differentiated; adeno: adenocarcinoma; ca: carcinoma.

TABLE 3. Breast Carcinomas

Tumor	Sex/age	Location	Histologic type	Previous treatment	Nude passages	Refs.
CLO	F/30	Left breast primary	Inf. duct cell ca	None	53	
KIE	F/51	Cell Line pleural effusate	Inf. duct cell ca	5-FU	22	(21)
WAR	F/62	Chest wall recurr.	Inf. duct cell ca	Rx	24	
VAN	F/49	Pleural effusate	Inf. duct cell ca	CTX, AL, Adria, 5-FU, MTX	25	
DRE	F/65	Right breast primary	Inf. duct cell ca	None	17	(21)
ELL	F/32	Breast primary	Inf. duct cell ca	None	24	
HIG	M/78	Right chest wall metastasis	Inf. duct cell ca	?	27	
COO	F/?	Cell line	Inf. duct cell ca	?	12	
ALL	F/66	Left breast primary	Medullary ca	None	8	
HUR	F/63	Brain metastasis cell line	Inf. duct cell ca	Multiple chemother	5	(3)
MUR	F/40	Left breast primary	Inf. duct cell ca	None	13	
JAM	F/43	Left ax. lymph node	Inf. duct cell ca	*C. parvum*, 5-FU, CTX, MTX, VC, Adria	4	
SAW	F/56	Right breast primary	Inf. duct cell ca	None	8	
WIS	F/48	Breast primary	Medullary ca	None	3	

Inf: infiltrating; ca: carcinoma; 5-FU: 5-fluorouracil; Rx: radiation therapy; CTX: Cytoxan (cyclophosphamide); AL: Alkeran (melphalan); Adria: Adriamycin; MTX: methotrexate; VC: vincristine.

fragment was carefully measured immediately after being positioned under the kidney capsule. A micrometric ocular inserted in a stereo microscope was used for this operation. The inoculated animals were then divided into groups of six to ten animals. Each day one control group was injected intraperitoneally with saline, 0.1 ml. The other groups were treated daily with chemotherapeutic agents at the doses shown in Table 5. The treatment was initiated when the implants were well established, having roughly doubled their initial mass. This was determined by killing inoculated animals every second day until doubling was confirmed. The mice were then injected daily for eight days, killed, and the tumors measured again. The tumor mass was calculated according to the formula

$$\text{Weight (mg)} = \frac{a \times b^2}{2}$$

where a is the length of the tumor and b is the width. Tumor growth inhibition was calculated by comparing the mass of the treated and untreated tumors. A strong inhibition (++) was arbitrarily assessed when the treated tumor mass was reduced 99% or more in comparison with the untreated tumor which served as a control. A moderate (+) inhibition was assessed with a reduction in tumor mass of more than 80% but less than 99%. Any reduction in tumor mass of less than 80% was considered a negative (−) result. Examples of results obtained in two typical experiments are shown in Figures 1 and 2.

TABLE 4. Summary of Patients/Tumors in Study

	Melanomas	Colorectal carcinomas	Breast carcinomas
Age (avg/range)	39 (21–55)	57 (41–81)	51 (30–78)
Sex	M:8, F:6	M: 8, F: 6	M: 1, F: 13
Passages	1–25	2–36	2–29

TABLE 5. Drug Doses in Panel Studies

Adriamycin	1 mg/kg/day × 8
5-FU	20 mg/kg/day × 8
Methotrexate*	8 mg/kg/day × 8
Cytoxan	20 mg/kg/day × 8
Alkeran	1 mg/kg/day × 8
Vincristine	0.2 mg/kg/day × 8
Vinblastine	0.3 mg/kg/day × 8
Methyl-CCNU	0.4 mg/kg/day × 8
BCNU	0.4 mg/kg/day × 8

* Initially MTX was given in doses of 24 mg/kg/day × 8. This was gradually scaled down to 8 mg/kg/day × 8 due to toxicity.

Results

Our results are reported in Tables 6 through 8. Because the clinical reports on the effectiveness of a drug in the treatment of a given malignancy do not always agree among themselves, we decided to report more than one clinical series for each type of tumor studied. We have, accordingly, compared our results with the results reported in some recent clinical compilations chosen on the basis of the number of patients reported and on the quantitation of the reports. It must be remembered that we are considering effective only those anticancer agents that when administered alone give a positive response in 20% or more of the treated patients. Consequently, we cannot use for comparison any reports that do not give the exact percentage of positive responders.

The results of our series and of the clinical series we used for comparison are tabulated side by side in Tables 9 through 11. When the results were borderline, either in our series or in the clinical series, the data are ex-

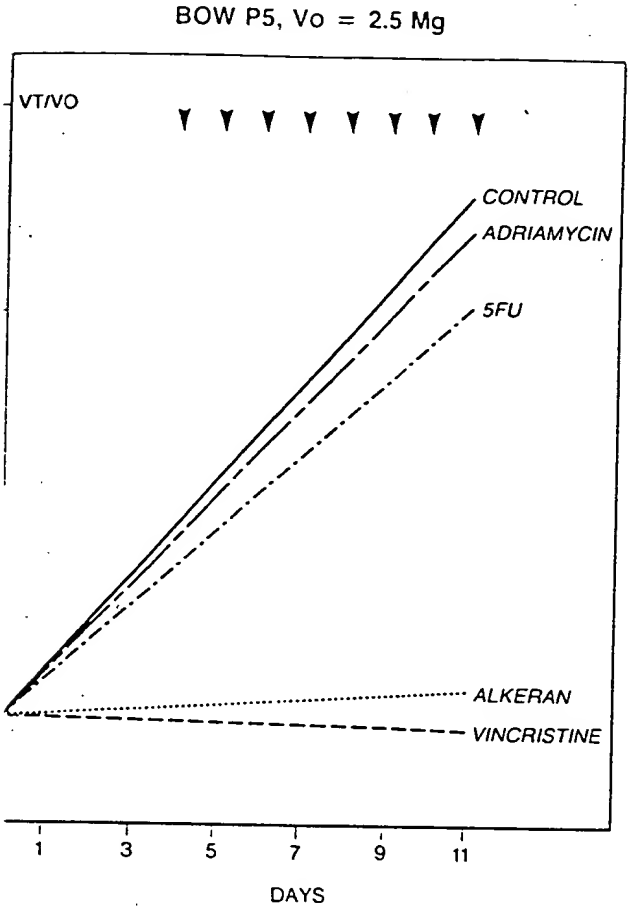

FIG. 1. Results obtained in typical experiment for melanoma.

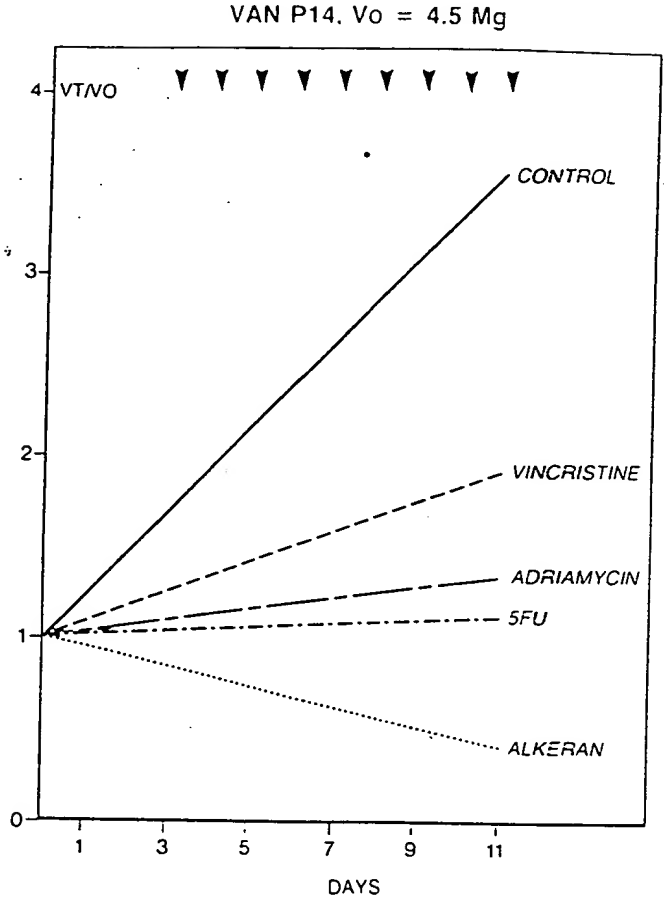

FIG. 2. Results obtained in typical experiment for breast carcinoma.

ssed as the real percentage in parentheses and are ntioned as being borderline in the general summary ble 12).

This is not the place for a morphologic description he tumors used. However, some observations made during this study may help one understand the results obtained. As reported in Tables 1 through 4, the melanomas and the colorectal carcinomas used in the panels did not differ appreciably from the average tumor of these types found at random in the human population.

TABLE 6. Melanomas

Adria	5-FU	MTX	CTX	AL	VC	VB	Methyl-CCNU	BCNU
–	–	–	–	⊖	–	–	–	–
+	++	+	++	–	++	++	–	++
++	++	–	–	+	++	–	–	+
–	–	–	–	⊖	–	–	–	–
–	–	–	–	+	++	–	–	–
–	–	–	–	⊖	–	–	–	–
–	–	–	–	–	+	–	–	–
–	–	–	+	–	+	+		–
–	–	–	–	–	–	–	–	–
–	–	–	–	–	+	–	–	–
–	–	–	–	⊖	–	–	–	–
–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–
–	–	–	+	–	–	–	–	–

te: Circle indicates that the patient was treated with the drug in ion before or after biopsy was taken.

Adria: Adriamycin; MXT: methotrexate; CTX: Cytoxan; AL: Alkeran; VC: vincristine; VB: vinblastine.

TABLE 7. Colorectal Carcinomas

	Adria	5-FU	MTX	CTX	AL	VC	VB	Methyl-CCNU	BCNU
GOB	–	–	–	–	–	–	–	–	–
KLO	–	–	–	–	–	–	–	–	–
FEI	+	–	–	++	–	–	–	–	–
PEY	–	(++)	–	–	–	–	–	–	–
CAS	–	–	–	–	–	–	–	–	–
HT-29	–	–	–	–		–	–	–	–
BEG	–	–	–	–	–	–	–	–	–
SQU	–	⊖	–	–	–	–	–	–	–
KON	–	⊖	–	–	–	–	–	–	–
KEN	–	–	–	–	–	–	–	–	–
ANZ	–	–	–	–	–	–	–	–	–
MOR	–	–	+	–	–	–	–	–	–
NOV	–	–	–	–	–	–	–	–	–
WAL	–	–	–	–	–	–	–	–	++

Note: Circle indicates that the patient was treated with the drug in question before or after biopsy was taken.

Adria: Adriamycin; CTX: Cytoxan; AL: Alkeran; VC: vincristine; VB: vinblastine.

TABLE 8. Breast Carcinomas

	Adria	5-FU	MTX	CTX	AL	VC	VB	Methyl-CCNU	BCNU
CLO	–	+	–	++	++	++	++	–	–
KIE	–	–	–	+	++	++	++	–	–
WAR	+	++	+	–	+	+	+	–	–
VAN	+	+	–	+	++	–	++	–	–
DRE	–	–	–	–	–	–	–	–	–
ELL	–	–	–	–	–	–	–	–	–
HIG	–	–	+	–	–	–	–	–	–
COO	–	–	–	–	–	–	–	–	–
ALL	–	–	–	–	–	–	–	–	–
HUR	–	–	–	–	–	–	–	–	–
MUR	–	–	–	–	–	–	–	–	–
JAM	⊖	⊖	⊖	⊖	–	(++)	–	–	–
SAW	–	–	–	–	⊖	–	–	–	–
WIS	–	–	–	–	–	–	–	–	–

Note: Circle indicates that the patient was treated with the drug in question before or after biopsy was taken.

Adria: Adriamycin; MTX: methotrexate; CTX: Cytoxan; AL: Alkeran; VC: vincristine; VB: vinblastine.

TABLE 9. Results of Panel Studies (Nude Mice/Clinical Series) of Melanomas

	Nude mice	Comis, Carter[12]	Luce[14]
Adriamycin	+	–	–
5-FU	+	–	–
Methotrexate	–	–	–
Cytoxan	+	+	+
Alkeran	+*	+	–
Vincristine	+	–	+
Vinblastine	+	+†	+
Methyl-CCNU	–	–	–
BCNU	+	–	+‡

* 96–98% inhibition.
† 19% responders.
‡ 18% responders.
A positive (+) result on this table is the equivalent of a ++ result on Tables 6–8 and indicates 99% or more inhibition, with the exception noted.

That is, these tumors were no more and no less differentiated than 14 tumors of each type chosen at random in the human population.

Breast carcinomas, however, were present only as undifferentiated tumors.

Discussion

The first question that we have to answer is whether the tumors composing the panels are truly representative of the populations on which the clinical research is conducted. From the standpoint of a pathologist, the answer is that both the colorectal carcinomas and the melanomas used in the panels are good representatives of such tumors found in the human population in the United States. Where the discrepancy between the type

tumors prevailing in the panel and in the population large is quite striking is in the breast carcinomas. The mors in our panel are, without exception, very unfferentiated, whereas in the population at large, only)% of the total breast carcinomas would be so classid.[7] This discrepancy is caused by the biological bevior of human breast carcinomas heterotransplanted nude mice. Under optimal conditions from 60% to % of human colorectal carcinomas and melanomas n be successfully heterotransplanted in nude mice. owever, only about 20% of breast carcinomas can be transplanted (our unpublished data). The tumors that give positive takes all belong to the less differentiated tegory and, with few exceptions, do not possess estron receptors.

The vast majority of the tumors in the panels had not ceived previous chemotherapy of any type. In partic-ar, 10/14 melanomas, 10/14 colorectal carcinomas, d 8/14 breast carcinomas had not previously been ated with any of the drugs used in our investigations. A difficult choice has been the selection of the clinical ults with which we would compare our experimental ults. Not all the clinical trials in the literature agree ong themselves as to the usefulness of a given drug inst a certain type of cancer, nor do they agree on percentages of positive results, largely because of the ferent criteria used to assess the effectiveness of chetherapeutic agents. We are also limited by the fact t we required well defined quantitative results. We e chosen the most recent clinical compilations in ich precise percentages of responders were given and which most of the patients were residents of the US latter stipulation to minimize possible population iations).[9–16]

Because the various clinical series do not always agree ong themselves, we have reported the figures obed from different series separately. When all the clinseries agree about results, we consider the chemoapeutic agent effective or not effective against the n type of cancer. The clinical series being studied ed 21 times and disagreed 6 times. Taking into acnt the 21 cases of agreement, our results in the nude e agreed with the clinical consensus in 18 instances ctive drugs and 9 inactive drugs). There were two -positives (Adriamycin [doxorubicin] and 5-fluoacil [5-FU] in melanomas) and one false-negative riamycin in breast carcinomas).

Given the way the tests were conducted, it is not imible that the false positives represent tumors that y are sensitive to the drug tested. To clarify this, one t remember that the concept of responsiveness is a ive and necessarily arbitrary one. We have arbitrarily decided that 20% of the tumors must respond in order to classify the tumor type as responsive to a given drug. Even if we keep our limits reasonably elastic, 15% to 18% might still be considered positive, but 5% to 10% would certainly not be. However, 5% to 10% still gives one responding tumor in every 10 to 20, an infrequent but not impossible occurrence, especially when a group of 14 tumors is being considered.

TABLE 10. Results of Panel Studies (Nude Mice/Clinical Series) of Colorectal Carcinomas

	Nude mice	Carter, Friedman[10]	Cline, Haskell[11]	Smith *et al.*[16]
Adriamycin	–	–	–	–
5-FU	+	+	+	+
Methotrexate	–	–	–	–
Cytoxan	+	+	–	–
Alkeran	–	–	–	–
Vincristine	–	–	–	–
Vinblastine	–	–	–	–
Methyl-CCNU	–	–	–	–
BCNU	+	+*	–	–

*15% responders.

A positive (+) result on this table is the equivalent of a ++ result on Tables 6–8 and indicates 99% or more inhibition.

TABLE 11. Results of Panel Studies (Nude Mice/Clinical Series) of Breast Carcinomas

	Nude mice	Carter[9]	Davis, Carbone[13] mod. by Rubens[15]
Adriamycin	–	+	+
5-FU	+	+	+
Methotrexate	+*	+	+
Cytoxan	+	+	+
Alkeran	+	+	+
Vincristine	+	+	+
Vinblastine	+	+	+
Methyl-CCNU	–	–	–
BCNU	–	–	+

* 96% and 92% inhibition.

A positive result (+) on this table is the equivalent of a ++ result on Tables 6–8 and, with the exceptions noted, indicates 99% or more inhibition.

TABLE 12. Correlations

Breast carcinomas	6/7 Positives*
	0 False-positives
Colorectal carcinomas	1/1 Positive
	0 False-Positive
Melanomas	2/2 Positives†
	2 False-Positives‡

* Methotrexate is borderline in nude mice test.

† Vinblastine is borderline in Comis & Carter series.

‡ BCNU is borderline in Luce series.

More disturbing is the existence of the false-negative, particularly of a drug such as Adriamycin in breast carcinoma, for Adriamycin is undoubtedly active against at least 30% of these neoplasms. As we have stated, breast carcinoma has limited percentage of positive takes (approximately 20%) in heterotransplanting in nude mice. Having selected a defined subclass of breast carcinomas that comprises about 20% of such tumors, it is not inconceivable that we would find such a class resistant to a drug that is ineffective against 60% or more of breast carcinomas.

Considering the large number of human tumors studied, the close correlation between results observed clinically and those obtained experimentally makes it difficult to avoid the conclusion that the human tumor heterotransplanted in the nude mouse is a good predictor of the results to be expected in the clinical setting. Such parallelism is further strengthened by the episodic cases in which the same human tumor has been subjected to the same treatment in the mice and in the patient with the same results.

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